Effect of adjuvant Shuxuening injection therapy on the inflammatory response mediated by TLRs in patients with acute exacerbation of COPD

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ABSTRACT

Objective: To study the effect of adjuvant Shuxuening injection therapy on the inflammatory response mediated by TLRs in patients with acute exacerbation of COPD. Methods: Patients with acute exacerbation of COPD who received Shuxuening injection combined with conventional western medicine treatment in our hospital between May 2014 and December 2015 were selected for study, and before treatment as well as 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, peripheral blood mononuclear cells were collected to determine TLRs expression, and serum was collected to determine the levels of inflammatory cytokines and airway remodeling molecules. Results: 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, TLR2 and TLR4 mRNA expression in peripheral blood mononuclear cells as well as TNF-α, IL-6, MCP-1, VCAM-1, MMP2, MMP9, TGF-β and BMP-2 levels in serum of patients with COPD were significantly lower than those before treatment, and TLR3, TLR7, TLR8 and TLR9 mRNA expression were not significantly different from those before treatment; TLR2 and TLR4 mRNA expression in peripheral blood mononuclear cells were positively correlated with serum TNF-α, IL-6, MCP-1, VCAM-1, MMP2, MMP9, TGF-β and BMP-2 levels. Conclusion: Adjuvant Shuxuening injection therapy has significant inhibitory effect on the inflammatory response mediated by TLRs in patients with acute exacerbation of COPD.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the chronic respiratory disease characterized by incompletely reversible airway limitation, and the condition progressively develops[1]. Infection and other incentives will cause the development of stable COPD into acute exacerbation of COPD and then increase the risk of respiratory failure, systemic inflammatory response syndrome, multiple organ dysfunction and other severe complications, and severe cases may endanger the life security[2,3]. Shuxuening injection is a traditional Chinese medicine preparation that can relieve asthma and moisten lungs, and its combination with western medicine treatment of acute exacerbation of COPD can achieve exact curative effect. However, the functioning molecular mechanism of Shuxuening injection is not clear at present. TLRs are a family of important pattern recognition receptors in the body, and they are involved in the regulation of the inflammatory response and closely related to the development of COPD. In the following study, the effect of adjuvant Shuxuening injection therapy on the inflammatory response mediated by TLRs in patients with acute exacerbation of COPD was analyzed.

2. Subjects and methods

2.1 Research subjects

Patients with acute exacerbation of COPD who received Shuxuening injection combined with conventional western medicine treatment in our hospital between May 2014 and December 2015...
were selected for study, all patients conformed to the diagnostic criteria for acute exacerbation of COPD, and total 35 cases were included, including 22 male cases and 13 female cases that were 64-77 years old. After admitted in hospital, they received Shuxuening injection combined with conventional western medicine treatment, western medicine treatment included anti-infection, reducing phlegm, relieving asthma, maintaining water and electrolyte balance, etc., and Shuxuening injection treatment was as follows: adding 15 mL of Shuxuening injection in 250 mL of saline, intravenous drip, 2 times/d, for consecutive 28 d.

2.2 TLRs mRNA expression detection methods

Before treatment as well as 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, 5 mL of peripheral blood was collected from two groups of patients, added in lymphocyte separation medium and centrifuged to separate peripheral blood mononuclear cells and then wash with PBS and centrifuge them twice, the RNA extraction kits were used to extract the total RNA in mononuclear cells and then reverse-transcribe it into cDNA, and fluorescence quantitative PCR amplification was performed to detect TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9 mRNA expression.

2.3 Serum inflammatory cytokine and airway remodeling molecule level detection methods

Before treatment as well as 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, 5 mL of peripheral blood was collected from two groups of patients and centrifuged to separate serum, and then enzyme-linked immunosorbent assay kits were used to determine TNF-α, IL-6, MCP-1, VCAM-1, MMP2, MMP9, TGF-β and BMP-2 levels.

2.4 Statistical methods

SPSS 22.0 software was used to input and statistically process data, measurement data comparison before and after treatment was by variance analysis of repeated measures and correlation analysis was by Pearson test. *P<0.05 indicated statistical significance in differences.

Table 1.

Comparison of TLRs expression in peripheral blood mononuclear cells before and after treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>TLR2</th>
<th>TLR3</th>
<th>TLR4</th>
<th>TLR7</th>
<th>TLR8</th>
<th>TLR9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>35</td>
<td>1.03±0.15</td>
<td>1.05±0.13</td>
<td>1.02±0.13</td>
<td>1.04±0.12</td>
<td>1.06±0.17</td>
<td>1.08±0.13</td>
</tr>
<tr>
<td>1 week after treatment</td>
<td>35</td>
<td>0.89±0.17</td>
<td>1.02±0.14</td>
<td>0.86±0.09</td>
<td>1.05±0.14</td>
<td>1.02±0.13</td>
<td>1.03±0.15</td>
</tr>
<tr>
<td>2 weeks after treatment</td>
<td>35</td>
<td>0.74±0.08</td>
<td>0.98±0.11</td>
<td>0.72±0.08</td>
<td>0.98±0.11</td>
<td>0.98±0.14</td>
<td>0.98±0.11</td>
</tr>
<tr>
<td>3 weeks after treatment</td>
<td>35</td>
<td>0.57±0.07</td>
<td>1.04±0.16</td>
<td>0.48±0.07</td>
<td>1.02±0.14</td>
<td>0.96±0.12</td>
<td>1.02±0.13</td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>35</td>
<td>0.35±0.04</td>
<td>0.99±0.11</td>
<td>0.32±0.05</td>
<td>0.97±0.12</td>
<td>1.03±0.14</td>
<td>1.06±0.17</td>
</tr>
</tbody>
</table>

*: compared with before treatment, *P<0.05; *a: compared with 1 week after treatment, *P<0.05; *b: compared with 2 weeks after treatment, *P<0.05; *c: compared with 3 weeks after treatment, *P<0.05.

3. Results

3.1 TLRs expression in peripheral blood mononuclear cells before and after treatment

Analysis of TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9 mRNA expression in peripheral blood mononuclear cells before and after treatment was as follows: 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, TLR2 and TLR4 mRNA expression in peripheral blood mononuclear cells of patients with COPD were significantly lower than those before treatment (*P<0.05), and TLR3, TLR7, TLR8 and TLR9 mRNA expression were not significantly different from those before treatment (*P>0.05); 2 weeks, 3 weeks and 4 weeks after treatment, TLR2 and TLR4 mRNA expression in peripheral blood mononuclear cells of patients with COPD were significantly lower than those 1 week after treatment (*P<0.05); 3 weeks and 4 weeks after treatment, TLR2 and TLR4 mRNA expression in peripheral blood mononuclear cells of patients with COPD were significantly lower than those 2 weeks after treatment (*P<0.05); 4 weeks after treatment, TLR2 and TLR4 mRNA expression in peripheral blood mononuclear cells of patients with COPD were significantly lower than those 3 weeks after treatment (*P<0.05).

3.2 Serum inflammatory cytokine levels before and after treatment

Analysis of serum inflammatory cytokines TNF-α, IL-6, MCP-1 and VCAM-1 levels before and after treatment was as follows: 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, serum TNF-α, IL-6, MCP-1 and VCAM-1 levels of patients with COPD were significantly lower than those before treatment (*P<0.05); 2 weeks, 3 weeks and 4 weeks after treatment, serum TNF-α, IL-6, MCP-1 and VCAM-1 levels of patients with COPD were significantly lower than those 1 week after treatment (*P<0.05); 3 weeks and 4 weeks after treatment, serum TNF-α, IL-6, MCP-1 and VCAM-1 levels of patients with COPD were significantly lower than those 2 weeks after treatment (*P<0.05); 4 weeks after treatment, serum TNF-α, IL-6, MCP-1 and VCAM-1 levels of patients with COPD were significantly lower than those 3 weeks after treatment (*P<0.05).
Comparison of serum airway remodeling molecule levels before and after treatment (pg/mL).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>MMP2</th>
<th>MMP9</th>
<th>TGF-β</th>
<th>BMP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>35</td>
<td>65.96±7.76</td>
<td>77.69±8.14</td>
<td>53.68±7.82</td>
<td>37.64±5.68</td>
</tr>
<tr>
<td>1 week after treatment</td>
<td>35</td>
<td>51.34±5.75</td>
<td>62.57±8.23</td>
<td>41.54±5.76</td>
<td>30.14±3.92</td>
</tr>
<tr>
<td>2 weeks after treatment</td>
<td>35</td>
<td>40.55±5.61</td>
<td>51.35±6.74</td>
<td>33.24±4.97</td>
<td>22.42±3.14</td>
</tr>
<tr>
<td>3 weeks after treatment</td>
<td>35</td>
<td>32.46±4.47</td>
<td>37.79±4.65</td>
<td>25.71±3.32</td>
<td>17.65±2.58</td>
</tr>
<tr>
<td>4 weeks after treatment</td>
<td>35</td>
<td>25.24±3.16</td>
<td>30.14±4.03</td>
<td>19.58±2.58</td>
<td>12.32±1.53</td>
</tr>
</tbody>
</table>

*: compared with before treatment, \( P<0.05 \);  †: compared with 1 week after treatment, \( P<0.05 \); ‡: compared with 2 weeks after treatment, \( P<0.05 \); §: compared with 3 weeks after treatment, \( P<0.05 \).

3.3 Serum airway remodeling molecule levels before and after treatment

Analysis of serum airway remodeling molecules MMP2, MMP9, TGF-β and BMP-2 levels before and after treatment was as follows: 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, serum MMP2, MMP9, TGF-β and BMP-2 levels of patients with COPD were significantly lower than those before treatment \( (P<0.05) \); 2 weeks, 3 weeks and 4 weeks after treatment, serum MMP2, MMP9, TGF-β and BMP-2 levels of patients with COPD were significantly lower than those 1 week after treatment \( (P<0.05) \); 3 weeks and 4 weeks after treatment, serum MMP2, MMP9, TGF-β and BMP-2 levels of patients with COPD were significantly lower than those 2 weeks after treatment \( (P<0.05) \); 4 weeks after treatment, serum MMP2, MMP9, TGF-β and BMP-2 levels of patients with COPD were significantly lower than those 3 weeks after treatment \( (P<0.05) \).

3.4 Correlation of TLR2 and TLR4 expression with inflammatory cytokines and airway remodeling molecules

Pearson test analysis results of the correlation of TLR2 and TLR4 expression in peripheral blood mononuclear cells with serum levels of inflammatory cytokines and airway remodeling molecules were as follows: TLR2 and TLR4 mRNA expression in peripheral blood mononuclear cells were positively correlated with serum TNF-α, IL-6, MCP-1, VCAM-1, MMP2, MMP9, TGF-β and BMP-2 levels.
mRNA expression in mononuclear cells decreased in time-dependent manner. This means that adjuvant Shuxuening injection therapy can inhibit the TLR2 and TLR4 expression in patients with acute exacerbation of COPD.

TLR2 and TLR4 molecules in patients with acute exacerbation of COPD can activate NF-κB through adaptor molecule MyD88 and make it transfer into the nucleus, which starts the transcription process of a variety of inflammatory factors. TNF-α, IL-6, MCP-1 and VCAM-1 are the inflammatory factors regulated by TLR2 and TLR4. TNF-α changes first in the process of inflammatory response, and it can not only mediate inflammatory tissue damage, but also recruit inflammatory cells in local lesions and enlarge inflammatory response; IL-6 is with multiple biological activities and has regulating effect on the inflammatory response and immune response[12]; MCP-1 has promoting effect on the mononuclear macrophage infiltration to lesions; VCAM-1 can mediate neutrophil, eosinophil and mononuclear macrophage adhesion with vascular endothelium, thus promoting the infiltration of a variety of inflammatory cells within lesions.[13,14]. In the study, analysis of the serum cytokine levels before and after treatment showed that 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, serum TNF-α, IL-6, MCP-1 and VCAM-1 levels of patients with COPD were significantly lower than those before treatment and positively correlated with TLR2 and TLR4 mRNA expression. This means that adjuvant Shuxuening injection therapy can inhibit the expression of TLR2 and TLR4 to reduce the secretion and release of inflammatory cytokines.

In the development and change of acute exacerbation of COPD, excessively activated inflammation can cause the changes in the levels of a variety of proteases and cytokines and lead to airway remodeling, which affect airway function. Studies have confirmed that MMP2, MMP9, TGF-β and BMP-2 are closely related to the airway remodeling process in patients with COPD and patients with asthma. MMP2 and MMP9 can cause the degradation and structure remodeling of a variety of elements in airway wall matrix[15,16]; both TGF-β and BMP-2 are the important members of the TGF-β family, and have regulating effect on tissue repair and remodeling[17]. In the study, analysis of the serum airway remodeling molecule levels before and after treatment showed that 1 week, 2 weeks, 3 weeks and 4 weeks after treatment, serum MMP2, MMP9, TGF-β and BMP-2 levels of patients with COPD were significantly lower than those before treatment and positively correlated with TLR2 and TLR4 mRNA expression. This means that adjuvant Shuxuening injection therapy can inhibit the expression of TLR2 and TLR4 to inhibit the airway remodeling in patients with acute exacerbation of COPD.

To sum up, it is believed that adjuvant Shuxuening injection therapy can inhibit the TLR2 and TLR4 expression in patients with acute exacerbation of COPD, and thus reduce the inflammatory response mediated by TLR2 and TLR4.

References


